

AMENDMENT

In the Claims

The following Listing of Claims, will replace all prior listings, and versions, of claims in the application.

Listing of Claims

Claim 1. (original) A substrate compound comprising a hydrophobic moiety capable of integrating the compound into a micelle, a fluorescent moiety and an enzyme recognition moiety.

Claim 2. (original) The substrate compound of Claim 1 which has a net neutral charge in aqueous solution at a pH of about pH 8.

Claim 3. (original) The substrate compound of Claim 1 in which the enzyme recognition moiety comprises a protein kinase recognition sequence including at least one unphosphorylated residue capable of being phosphorylated by a protein kinase.

Claim 4. (original) The substrate compound of Claim 3 in which the at least one unphosphorylated residue is tyrosine, serine or threonine.

Claim 5. (original) The substrate compound of Claim 3 in which the protein kinase recognition sequence is recognized by a TK kinase, an AGC kinase, a CAMK kinase, a CMGC kinase, an STE kinase, a TKL kinase, a CKI kinase or a kinase belonging to the group "other."

Claim 6. (currently amended) The substrate compound of Claim 3 in which the protein kinase recognition sequence is recognized by a protein kinase A, a protein kinase C, a Src kinase, a Lyn kinase, a Fyn kinase, an Akt kinase, a MAP kinase a MAPKAP2 kinase or a cAMP dependent kinase.

Claim 7. (original) The substrate compound of Claim 3 in which the protein kinase recognition sequence comprises a peptide sequence selected from the group consisting of:

-R-R-X-S/T-Z-	(SEQ. ID NO:1);
-R-X-X-S/T-F-F-	(SEQ. ID NO:2);
-S/T-P-X-R/K-	(SEQ. ID NO:3);
-P-X-S/T-P-	(SEQ. ID NO:4);
-K-K-K-K-R-F-S-F-K-	(SEQ. ID NO:5);
-X-R-X-X-S-X-R-X-	(SEQ. ID NO:6);
-L-R-R-L-S-D-S-N-F-	(SEQ. ID NO:7);
-K-K-L-N-R-T-L-T-V-A-	(SEQ. ID NO:8);
-E-E-I-Y-E/G-X-F-	(SEQ. ID NO:9);
-E-I-Y-E-X-I/V-	(SEQ. ID NO:10);
-I-Y-M-F-F-F-	(SEQ. ID NO:11);
-Y-M-M-M-	(SEQ. ID NO:12);
-E-E-E-Y-F-	(SEQ. ID NO:13);
-L-R-R-A-S-L-G-	(SEQ. ID NO:14);
-R-Q-G-S-F-R-A-	(SEQ. ID NO:15);
-R-I-G-E-G-T-Y-G-V-V-R-R-	(SEQ. ID NO:16);
-R-P-R-T-S-S-F-	(SEQ. ID NO:17);
-P-R-T-P-G-G-R-	(SEQ. ID NO:18);

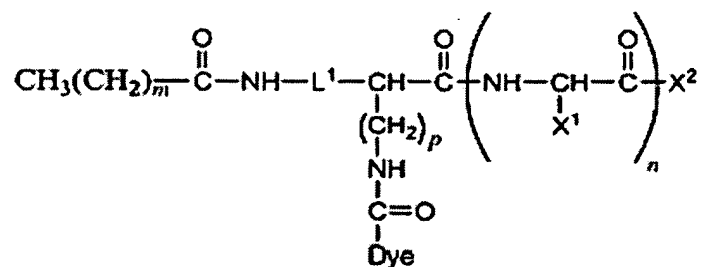
-R-L-N-R-T-L-S-V-

(SEQ. ID NO:19); and

analogues and conservative mutants thereof, wherein X represents any residue and Z represents a hydrophobic residue.

Claim 8. (original) The substrate compound of Claim 3 which has a net neutral charge in aqueous solution at a pH of about pH 8.

Claim 9. (original) The substrate compound of Claim 3 which has the structure:



wherein:

m is an integer from 4 to 28;

n is an integer from 3 to 15;

p is an integer from 1 to 6;

L¹ is an optional linker;

Dye is a fluorescent dye which optionally includes a linker linking the Dye to the illustrated adjacent carbonyl group;

each X¹ is, independently of the others, an amino acid side chain; and

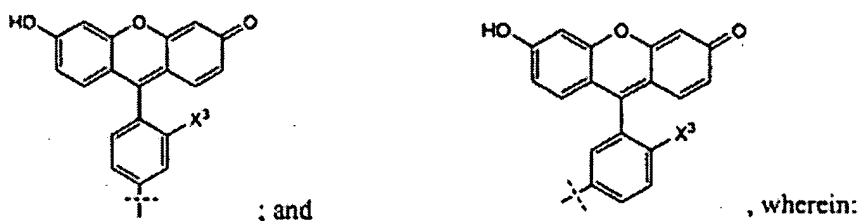
X² is OR or NH₂, where R is hydrogen or an alkyl containing from 1 to 8 carbon atoms,

with the proviso that the illustrated $-\text{[NH-CH(X}^1\text{)C(O)]}_n\text{-X}^2$ portion of the substrate compound includes at least one residue that is capable of being phosphorylated by a protein kinase.

Claim 10. (original) The substrate compound of Claim 9 in which L^1 is $-\text{[CH}_2\text{CH}_2\text{-O-CH}_2\text{CH}_2\text{-O-CH}_2\text{C(O)NH]}_q\text{-}$, where q is 0, 1, 2 or 3.

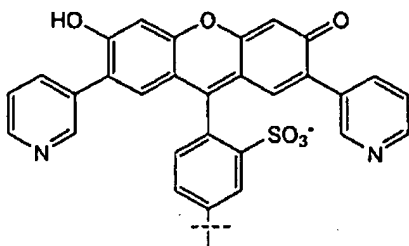
Claim 11. (original) The substrate compound of Claim 9 in which Dye comprises a fluorescein or a rhodamine dye.

Claim 12. (original) The substrate compound of Claim 11 in which Dye comprises an optionally substituted structure selected from:



X^3 is $-\text{C(O)O}^-$ or $-\text{SO}_3^-$ and the broken line indicates the point of attachment to the remainder of the illustrated structure.

Claim 13. (original) The substrate compound of Claim 12 in which Dye has the structure Dye2:



Claim 14. (currently amended) The substrate compound of Claim 9 in which the illustrated $-\text{[NH-CH(X}^1\text{)C(O)]}_n-$ portion of the substrate compound is a peptide is selected from the group consisting of:

-R-R-X-S/T-Z- (SEQ. ID NO:1);

-R-X-X-S/T-F-F- (SEQ. ID NO:2);

-S/T-P-X-R/K- (SEQ. ID NO:3);

-P-X-S/T-P- (SEQ. ID NO:4);

-K-K-K-K-R-F-S-F-K- (SEQ. ID NO:5);

-X-R-X-X-S-X-R-X- (SEQ. ID NO:6);

-L-R-R-L-S-D-S-N-F- (SEQ. ID NO:7);

-K-K-L-N-R-T-L-T-V-A- (SEQ. ID NO:8);

-E-E-I-Y-E/G-X-F- (SEQ. ID NO:9);

-E-I-Y-E-X-I/V- (SEQ. ID NO:10);

-I-Y-M-F-F-F- (SEQ. ID NO:11);

-Y-M-M-M- (SEQ. ID NO:12);

<u>-E-E-E-Y-F-</u>	(SEQ. ID NO:13);
LRRASLG	(SEQ ID NO:14);
RQGSFRA	(SEQ ID NO:15);
RIGEGTYGVVRR	(SEQ ID NO:16);
RPRTSSF	(SEQ ID NO:17);
PRTPGGR	(SEQ ID NO:18); and
RLNRTLVS	(SEQ ID NO:19).

Claim 15. (original) The substrate compound of Claim 3 in which the hydrophobic moiety comprises a substituted or unsubstituted, saturated or unsaturated hydrocarbon having from 6 to 30 carbon atoms.

Claim 16. (original) The substrate compound of Claim 15 in which the hydrocarbon is a linear, branched or cyclic, saturated or unsaturated alkyl.

Claim 17. (original) The substrate compound of Claim 16 in which the hydrocarbon is a linear alkyl containing from 10 to 26 carbon atoms.

Claim 18. (original) The substrate compound of Claim 17 in which the alkyl is fully saturated *n*-alkanyl.

Claim 19. (original) The substrate compound of Claim 17 in which the alkyl includes one or more carbon-carbon double bonds, each of which may, independently of the others, be in the *cis* or *trans* configuration and/or one or more carbon-carbon triple bonds.

Claim 20. (original) The substrate compound of Claim 3 in which the hydrophobic moiety contains at least one positively charged group.

Claim 21. (original) The substrate compound of Claim 3 in which the hydrophobic moiety contains at least one negatively charged group.

Claim 22. (original) The substrate compound of Claim 3 in which the fluorescent moiety comprises a dye selected from a xanthene dye, a rhodamine dye, a fluorescein dye, a cyanine dye, a phthalocyanine dye, a squaraine dye and a bodipy dye.

Claim 23. (original) The substrate compound of Claim 3 in which the fluorescent moiety comprises a fluorescence donor moiety and a fluorescence acceptor moiety.

Claim 24. (original) The substrate compound of Claim 23 in which the fluorescence donor moiety comprises a fluorescein dye.

Claim 25. (original) The substrate compound of Claim 23 in which the fluorescence acceptor moiety comprises a fluorescein or a rhodamine dye.

Claim 26. (original) The substrate compound of Claim 25 in which the fluorescence donor moiety comprises a fluorescein dye.

Claim 27. (original) The substrate compound of Claim 3 in which the fluorescent moiety comprises fewer than 150 atoms.

Claim 28. (original) The substrate compound of Claim 3 in which the hydrophobic moiety and the enzyme recognition moiety are linked to one another through the fluorescent moiety.

Claim 29. (original) The substrate compound of Claim 3 in which the hydrophobic moiety and the fluorescent moiety are linked to one another through the enzyme recognition moiety.

Claim 30. (original) The substrate compound of Claim 3 in which the hydrophobic moiety, the fluorescent moiety and the enzyme recognition moiety are linked to one another *via* a trivalent linker.

Claim 31. (original) The substrate compound of Claim 3 in which the hydrophobic moiety is linked to the fluorescent moiety by a linker than does not include a part of the enzyme recognition moiety.

Claim 32. (original) The substrate compound of Claim 3 in which the hydrophobic moiety is linked to the fluorescent moiety by a linker that includes at least a part of the enzyme recognition moiety.

Claim 33. (original) The substrate compound of Claim 1 in which the enzyme recognition moiety comprises a phosphatase recognition sequence including at least one phosphorylated residue capable of being dephosphorylated by a phosphatase.

Claim 34. (original) The substrate compound of Claim 33 which has a net neutral charge in aqueous solution at a pH of about pH 8.

Claim 35. (original) A method of detecting the presence of an enzyme activity in a sample, comprising the steps of:

contacting the sample with a composition comprising a substrate compound according to Claim 1 in which the enzyme recognition moiety is recognized by the enzyme, under conditions effective to permit the enzyme, when present in the sample, to modify the substrate compound in a manner that leads to an increase in a fluorescence signal produced by its fluorescent moiety; and

detecting a fluorescence signal, where an increase in the fluorescence signal indicates the presence and/or quantity of the enzyme in the sample.

Claim 36. (original) The method of Claim 35 in which the substrate compound is present at a concentration at or above its critical micelle concentration.

Claim 37. (original) The method of Claim 35 in which the fluorescence signal is detected as a function of time.

Claim 38. (original) The method of Claim 35 in which the composition further comprises a quenching compound which comprises a hydrophobic moiety capable of integrating the quenching compound into a micelle and a quenching moiety capable of quenching the fluorescence of the fluorescent moiety of the substrate compound.

Claim 39. (original) The method of Claim 35 which further comprises determining a K_m value or K_{cat} value for an enzyme in the sample.

Claim 40. (original) A method of identifying a compound that modulates an activity of an enzyme, comprising the steps of:

contacting the enzyme with a composition comprising a substrate compound according to Claim 1 in which the enzyme recognition moiety is recognized by the enzyme in the presence of a candidate modulator compound and under conditions effective to permit the enzyme allow the enzyme to modify the substrate compound in a manner that leads to an increase in a fluorescence signal produced by its fluorescent moiety; and

detecting a fluorescence signal, where an increase or decrease in the fluorescence signal as compared to a control reaction or a standard curve indicates that the candidate modulator compound modulates the activity of the enzyme.

Claim 41. (original) The method of Claim 40 in which the candidate modulator compound is a known modulator of the enzyme activity and the method is used to assess the effect of the modulator compound on the activity of the enzyme.

Claim 42. (original) The method of Claim 40 in which is carried out to identify an inhibitor of the enzyme activity, where a decrease in the fluorescence signal as compared to a control reaction or a standard curve indicates that the candidate modulator compound inhibits the activity of the enzyme.

Claim 43. (original) The method of Claim 42 which further comprises determining the K_i of the inhibitor compound.

Claim 44. (original) The method of Claim 42 in which the candidate modulator compound is a known inhibitor of the activity of the enzyme and the method is used to determine the K_i of the compound.

Claim 45. (original) A method of detecting phosphorylation activity of one or more protein kinases in a sample, comprising the steps of:

contacting the sample with a composition comprising a protein kinase substrate which comprises (1) a protein kinase recognition moiety containing at least one unphosphorylated residue capable of being phosphorylated by a protein kinase, (2) a hydrophobic moiety capable of integrating the substrate into a micelle, and (3) a fluorescent moiety, under conditions effective to allow phosphorylation of said residue when the protein kinase is present in the sample, thereby increasing a fluorescence signal produced by the fluorescent moiety; and

detecting a fluorescence signal, where an increase in the fluorescence signal indicates the presence and/or quantity of protein kinase phosphorylation activity in the sample.

Claim 46. (original) The method of Claim 45 in which the protein kinase substrate is a substrate compound according to any one of Claims 3-32.

Claim 47. (original) The method of Claim 45 in which the fluorescence signal is detected as a function of time.

Claim 48. (original) The method of Claim 45 in which the composition further comprises a quenching compound which comprises a hydrophobic moiety capable of integrating the quenching compound into a micelle and a quenching moiety capable of quenching the fluorescence of the fluorescent moiety of the protein kinase substrate.

Claim 49. (original) The method of Claim 45 which further comprises determining a K_m value or K_{cat} value for a protein kinase in the sample.

Claim 50. (original) A method of identifying a compound that modulates phosphorylation activity of a protein kinase, comprising the steps of:

contacting the protein kinase with a composition comprising a protein kinase substrate which comprises (1) a protein kinase recognition moiety containing at least one unphosphorylated residue capable of being phosphorylated by a protein kinase, (2) a hydrophobic moiety capable of integrating the substrate into a micelle, and (3) a fluorescent moiety, in the presence of a candidate compound and under conditions effective to allow phosphorylation of said residue by the protein kinase, thereby increasing a fluorescence signal produced by the fluorescent moiety; and

detecting a fluorescence signal, where an increase or decrease in the fluorescence signal as compared to a control reaction or a standard curve indicates that the candidate compound modulates the activity of the protein kinase.

Claim 51. (original) The method of Claim 50 in which the candidate compound is a known modulator of the protein kinase phosphorylation activity and the method is used to assess the effect of the compound on the phosphorylation activity of the protein kinase.

Claim 52. (original) The method of Claim 50 in which is carried out to identify an inhibitor of the protein kinase phosphorylation activity, where a decrease in the fluorescence signal as compared to a control reaction or a standard curve indicates that the candidate compound inhibits the phosphorylation activity of the protein kinase.

Claim 53. (original) The method of Claim 50 which further comprises determining the K_i of the inhibitor compound.

Claim 54. (original) The method of Claim 50 in which the candidate compound is a known inhibitor of the activity of phosphorylation activity the protein kinase and the method is used to determine the K_i of the compound.

Claim 55. (original) The method of Claim 50 in which the composition further comprises a quenching compound which comprises a hydrophobic moiety capable of integrating the quenching compound into a micelle and a quenching moiety capable of quenching the fluorescence of the fluorescent moiety of the protein kinase substrate.